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Electron microscopy of the complexes of ribulose-1,5-bisphosphate carboxylase (Rubisco) and Rubisco subunit-binding protein from pea leaves

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The structure of ribulose-1,5-bisphosphate carboxylase (Rubisco) subunit-binding protein and its interaction with pea leaf chloroplast Rubisco were studied by electron microscopy and image analysis. Electron-microscopic evidence for the association of Rubisco subunit-binding protein, consisting of 14 subunits arranged with 72 point group symmetry, and oligomeric (L_8S_8) Rubisco was obtained.

Ribulose-1,5-bisphosphate carboxylase (Rubisco); Rubisco subunit-binding protein; Quaternary structure; Protein-protein complex; Electron microscopy

1. INTRODUCTION

D-ribulose-1,5-bisphosphate carboxylase-oxygenase (EC 4.1.1.39) (Rubisco) is the key enzyme of photosynthetic inorganic carbon fixation. High plant Rubisco (molecular mass about 550 kDa) consists of 8 large and 8 small subunits (L_8S_8) which are synthesized in chloroplasts and the cytosol of a plant cell respectively [1]. Oligomeric structure of Rubisco studied by electron microscopy and X-ray crystallography, are shown to consist of eight LS-monomers arranged with 422 (D_4) symmetry in 2 layers with a diameter of the molecule of about 12.5 nm and a height of about 10 nm [2–4].

There are some biochemical data on the involvement of Rubisco subunit-binding protein in the post-translational folding and assembly of Rubisco subunits in chloroplasts [5,6]. The Rubisco subunit-binding protein, so-called because of its association with newly synthesized Rubisco subunits [5–7], corresponded to a GroEL-like family of proteins or chaperonins [8–10]. These proteins are considered to assist in assembly of polypeptides into their oligomeric form but they are not a part of the final structure. In bacteria and mitochondria the chaperonins (not only GroEL-like) take part in the refolding of polypeptide chains of subunits of some proteins after heat shock and formation of their correct oligomeric structure [11–14]. The groEL-like chaperonins have a very similar amino acid sequence [8] and the same quater-

nary structure with 14 subunits arranged in two layers [15–18]. The transient association of groEL-like chaperonins to unfolded proteins was observed during formation of dimeric Rubisco from *Rhodospirillum rubrum* in vitro [19], pre- β -lactamase and chloramphenicol acetyltransferase in *E. coli* [20], and Rubisco, β -subunit of ATP synthase, glutamine synthetase, and light-harvesting chlorophyll a/b binding protein of higher plants [21].

The facts of participation of Rubisco subunit-binding protein in the assembly of higher plant Rubisco allowed us to propose the possibility of a protein-protein interaction not only with unfolded subunits of Rubisco but also with the whole oligomeric molecule. The current work was performed to obtain direct evidence for the existence of such an interaction by electron microscopy. The quaternary structure of the Rubisco subunit-binding protein from pea leaf chloroplasts was also studied during this work.

2. MATERIALS AND METHODS

Rubisco and Rubisco subunit-binding protein were isolated from pea leaves according to [3] and [17] respectively.

For electron microscopy the protein preparations were negatively stained with 2% uranyl acetate. The grids were examined in a Philips EM 400 electron microscope at 80 kV with a magnification of 50000. The micrographs were digitized with a sampling corresponding 0.5 nm on the specimen level. Image analysis was carried out on Convex CI-XP mini-supercomputer with the IMAGIC software system [22]. Single particles for correlation averaging were selected interactively from the micrographs displayed on the monitor screen. A circular mask was imposed upon the particles to remove unnecessary background. The average density inside the mask was set to zero and the

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variance of density was normalized. The images to be averaged were aligned by the correlation method. As a first reference, a well-preserved particle was chosen. A few cycles of alignment and averaging were made with previous averages used as new references.

3. RESULTS AND DISCUSSION

Two distinct types of single particle projections of Rubisco subunit-binding protein could readily be recognized in the micrographs of preparations enriched with this protein (Fig. 1a). The first type (top view) has the form of 'seven-pointed' star of 14 ± 0.8 nm in diameter (Fig. 1b). The second type (side view) looks like a rectangle of 14.1 ± 1.0 nm length and 11.2 ± 0.7 nm width with four strips parallel to the short side (Fig. 1c). These two types are found more frequently than others and correspond most probably to stable positions of the

molecule on the support film. For more detailed analysis of molecular structure, the images of top view (Fig. 1b) and side view (Fig. 1c) were computer averaged by correlation method (see Section 2). The correlation analysis of individual particle images of front view (Fig. 1b) shows that they are characterized by 7-fold rotational symmetry, therefore this symmetry was imposed to each particle before the correlation averaging. After this procedure two groups of images with different handedness were obtained. This phenomenon may be due to preferential staining of back or front sides of the molecule. After mirror-symmetrization of the second group of images, all projections of this type had the same handedness. They were computer averaged as described in Section 2. The final image (7-fold rotational symmetrized) is shown in Fig. 2a. It has clear visible handedness with seven elongated stain-excluding

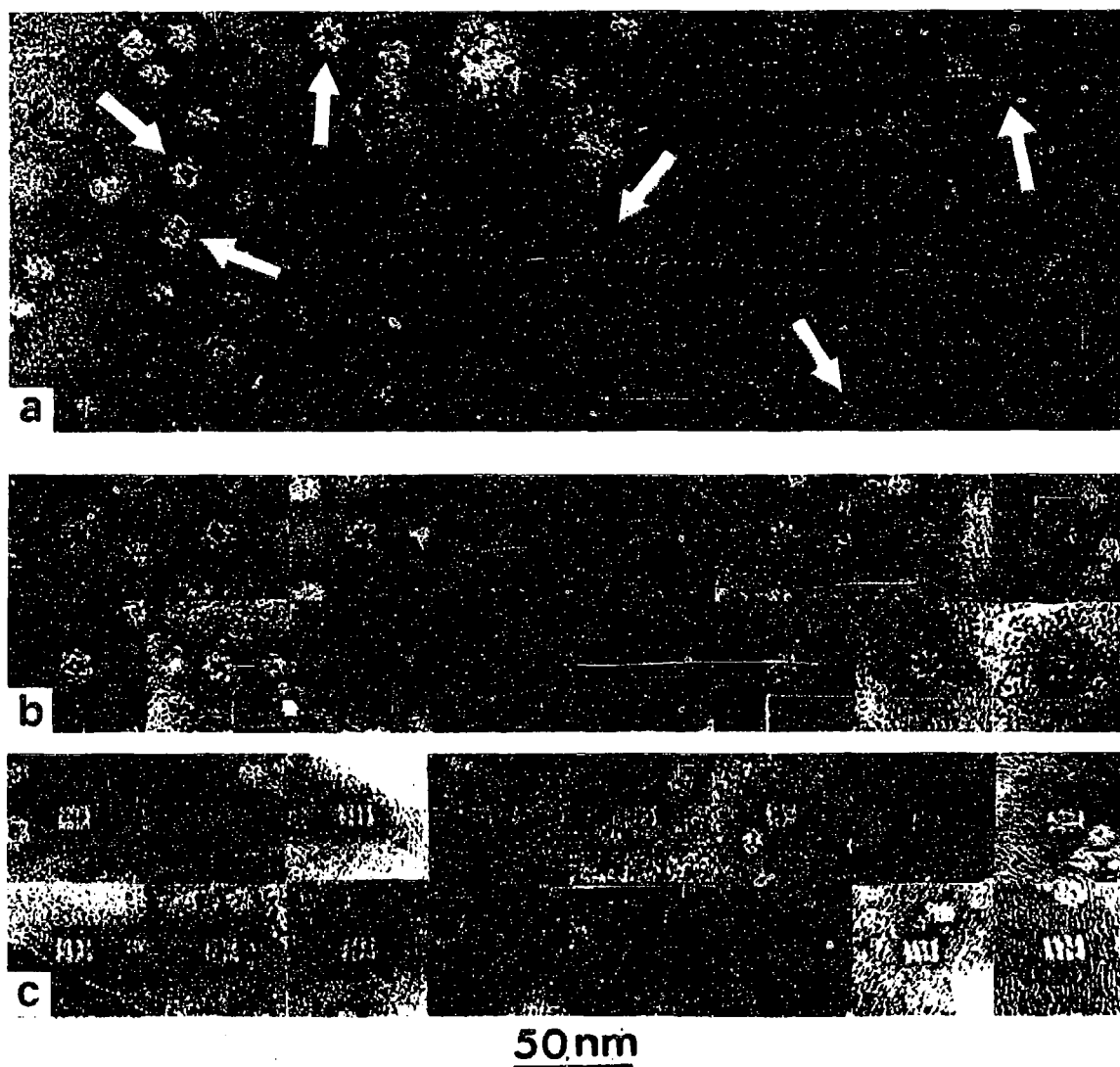


Fig. 1. (a) Electron micrograph of preparation of Rubisco subunit-binding protein from pea leaves negatively stained with uranyl acetate. Particles attached to the support in two different positions are indicated by arrows. (b,c) Selected molecules showing top (b) and side (c) view projections.

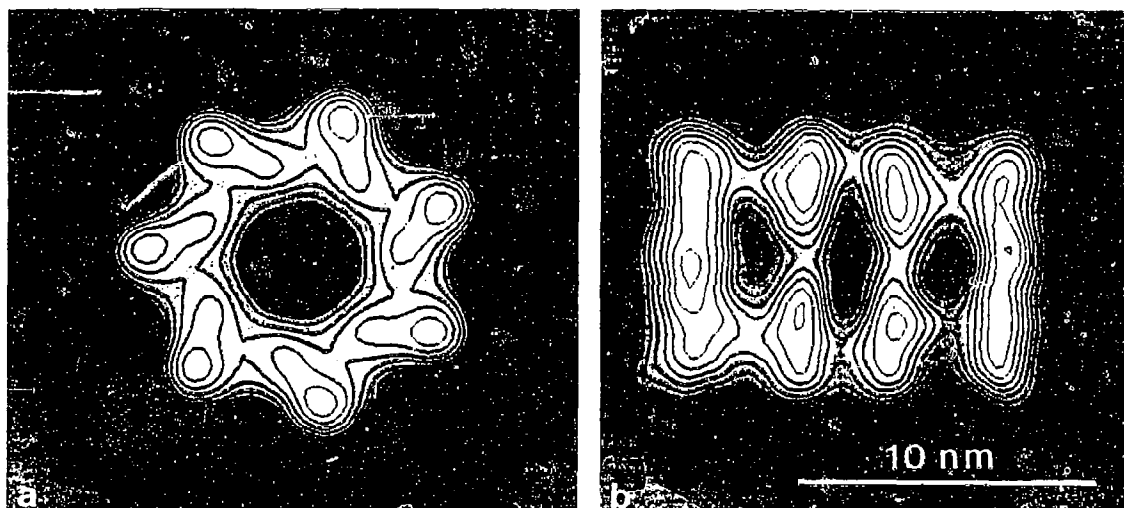


Fig. 2. Molecular projections of pea leaf Rubisco-subunit-binding protein. (a) Correlation averaging of images of top view (see Fig. 1b) with 7-fold symmetry imposed. (b) The average of images of the side view (see Fig. 1c).

(protein) regions. The result of averaging the images of the side view is shown in Fig. 2b. It divided with stain into 2 approximately equal parts which are related by mirror plane symmetry parallel to the short side of the rectangle. Taking into account that the molecule consists of very close α and β polypeptides in $\alpha_7\beta_7$ stoichiometry [23] the projections observed allowed us to suggest a model for the molecule: 14 subunits are arranged with 72 point group symmetry in two layers which are clearly visible in Fig. 2b. The presence of four protein strips in this projection oriented perpendicular to the 7-fold axis indicates that the molecule appears as a stalk of four discs (two discs in each layer) and each subunit may consist of two close-sized domains localized at different levels along the 7-fold axis. This conclusion is in agreement with data obtained earlier for heat shock protein from mitochondria [18]. The molecule has a diameter of about 14 nm and a height of about 14.1 nm. The length of the short side of the rectangular projection (Fig. 2b) is less than the diameter of the top view projection (Fig. 2a) that can be explained by partial staining of most particles in 'side-on' position. A very similar quaternary structure was also observed by electron microscopy for other chaperonins (groEL-like): groEL-protein of *E. coli* [15,16], high molecular weight protein of pea leaves [17] and heat shock protein of mitochondria [18]. Multicatalytic proteases or prosomes are another type of molecule with 7-fold symmetry in the top view and four striations in the side view revealed [24].

During this study we have found the existence of the complexes between Rubisco subunit-binding protein and the L_8S_8 Rubisco molecule. Fig. 3a shows the preparation of Rubisco containing small quantities of Rubisco subunit-binding protein. The main projection of Rubisco molecules is roundshaped of 12.5 ± 0.5 nm

in diameter. Eight protrusions grouped in pairs are seen over the particle circumference. These images can be interpreted as a projection of two tetramers (LS-promoters are arranged at the vertices of a square) only partially eclipsed down the 4-fold axis [3,4]. It is seen (Fig. 3a) that practically all molecules of Rubisco subunit-binding protein form complexes with the enzyme. The same result was obtained also after incubation of Rubisco with this protein.

We made an attempt to elucidate the way the protein and the enzyme are attached. Fig. 3 demonstrates the Rubisco subunit-binding protein in a complex. In the most cases one can see only two sites of the enzyme binding to the protein in its side projection: one or two Rubisco molecules contact with the protein in the middle of the short (Fig. 3b) or long (Fig. 3c) rectangle's sides. This indicates a specific manner of protein-enzyme binding. The main conclusion from these studies is that Rubisco and Rubisco subunit-binding protein can exist in an associated state and their complex is relatively stable in vitro. It should be mentioned that analogous complexes were found by us in preparations of higher plant glutamine synthetase which possesses very similar quaternary structure to higher plant Rubisco [25]. The nature of these complexes is not clear. It may be possible that chaperonins participate not only in folding and assembly of some enzymes but can associate with an enzyme oligomeric molecule to stabilize its structure.

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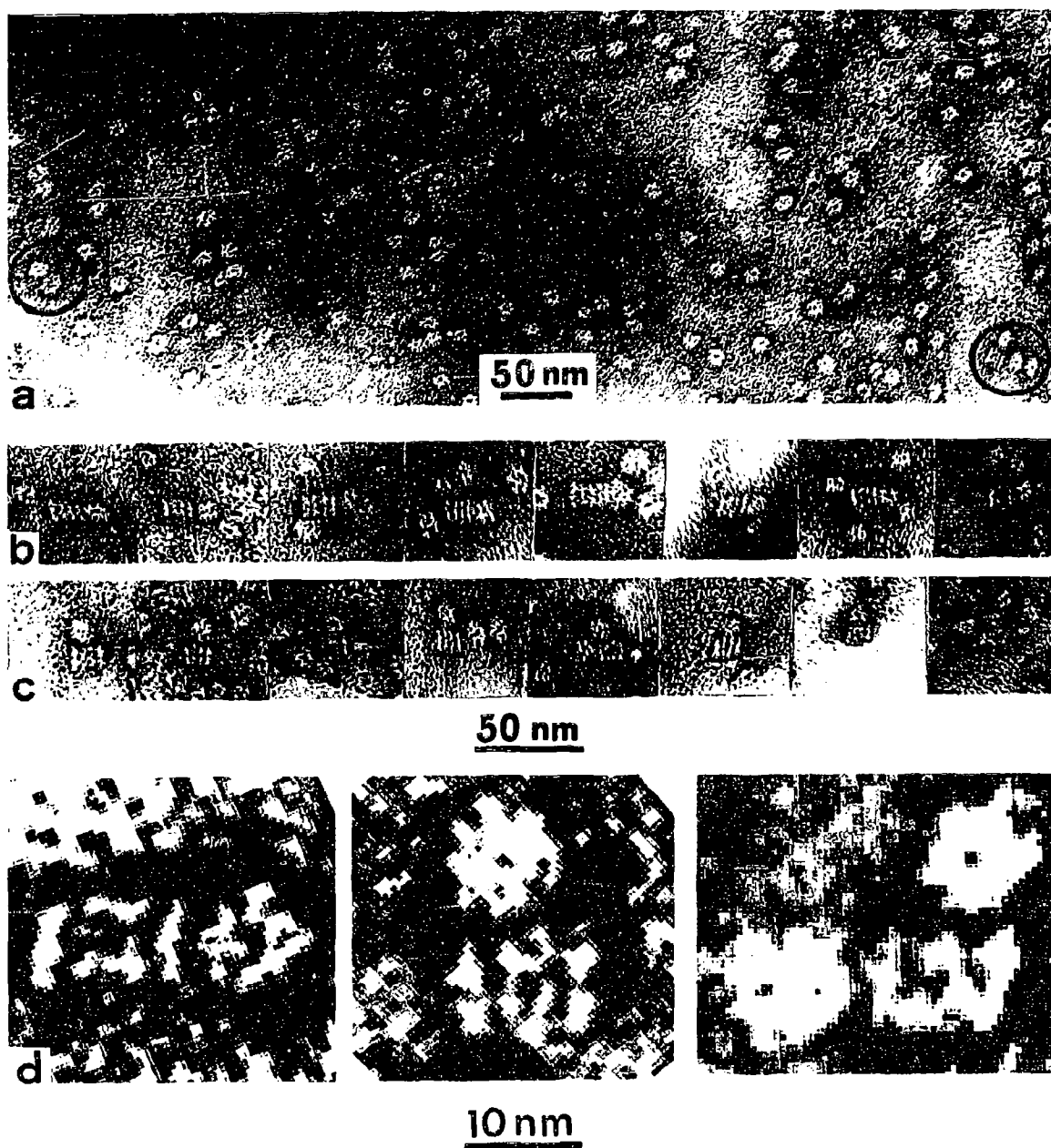


Fig. 3. (a) Electron microscopy of negatively stained preparations of pea leaf Rubisco and Rubisco subunit-binding protein. The protein-enzyme complexes are encircled. (b,c,d) Characteristic types of protein-enzyme complex images.

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